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Stability of Strains of Sugar Beet Yellows Virus and Influence of Host Genotype on the Symptoms

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Many severe, intermediate and mild isolates of sugar beet yellows virus (SBYV) originating from infected field plants were compared in serial transfers for several years — some for more than ten years. The isolates were found to reproduce with a high degree of stability. Repeated attempts to select and cultivate divergent types producing other symptoms than the original isolate have failed.

When the isolates of SBYV were tested on a number of plants of a commercial sugar beet variety, the most severe and the most mild isolates usually produced fairly uniform symptoms on the infected plants. Isolates of a moderately severe or intermediate type behaved more irregularly; on some of the test plants fairly severe and on some plants intermediate or more or less mild symptoms were produced. This seems to reflect different genetic symptom responses of different plants within the commercial sugar beet variety to the infection and not a heterogeneity of the virus isolates, as repeated transfers from plants with the most severe and from plants with the most mild reaction both produced the original range of symptoms on many successive series of test plants (Section IV of this paper). When such virus isolates were tested on moderately inbred sugar beets, they produced more uniform, but still slightly varying symptoms (Sections III and V). Almost identical symptoms, indicating an actual homogeneity of the virus isolates in question, were obtained when highly inbred sugar beet varieties or clonal lines of beets were infected (Sections V and VI).

I. A REVIEW OF DIFFERENT APHID TRANSMITTED BEET YELLOWING VIRUSES AND VIRUS STRAINS

A survey of the extensive literature dealing with this subject shows that not less than six apparently unrelated viruses or virus groups producing more or less pronounced yellowing of the beet leaves have been described.

1. *Sugar beet yellows virus* (SBYV). The disease caused by this virus is reported from most European countries (Rietberg 1959), from U.S.A. (Bennet 1960) and from Asia (Chiu et al. 1959). Different, serologically related strains are described (e.g. Watson 1951, Lüdecke, Schlösser & Nietsche 1956, Bennet, Price & Mc.Farlane 1957, Björling 1958, Burghardt & Bercks 1959, Hull 1959 Bennet 1960). The particles of this virus are filamentous (Leyon 1951).

2. *Sugar beet mild yellowing virus* (SBYMV). Isolates of this type are milder, have a longer incubation time and differ from SBYV in the following respects. They do not produce vein-etch symptoms, no filamentous particles have been found in sap from infected plants and they give no cross-protection against strains of true SBYV. On account of these properties Russel (1958. 1960) considers SBYMV as a separate virus.

3. *Radish yellows virus* (Duffus 1960). This virus is quite common in sugar beets in California and the symptoms are similar to those of SBYMV. However, the host range of radish yellows virus is substantially diffe-

rent from that of SBYV and SBYMV and it is probably a distinct virus.

4. *Seed-transmitted yellowing viruses.* The most wellknown case is 41-yellows virus (Clinch & Loughnane 1948, Watson, Hull & Hartsuijker 1949). A similar type was described by Hull (1955). These seed-borne viruses produce symptoms resembling those of SBYMV but they seem to have no relationship to No. 1—3.

5. *Yellow-net virus* (Sylvester 1948). This virus differs from the before mentioned ones in regard to symptoms, host range, serological properties and size and shape of the virus particles (Björling & Ossiannilsson 1958 a).

6. *Cucumber mosaic virus.* Some strains of Cucumis virus 1 produce systemic yellowing symptoms of sugar beets. Cases of field infection are described from California (Sylvester & Severin 1953).

The three last-mentioned of the six viruses cited above are uncommon as causes of diseases of sugar beets and only the three first-mentioned ones are of considerable economic importance to beet-growing. At present SBYV seems to be the most important one in Europe, at least on the Continent. However, in recent years also SBYMV has been reported to be very common in Great Britain (Russel 1958, 1960) and in Sweden too, virus isolates similar to SBYMV have been found in markedly increasing proportions since the middle fifties.

Varying symptoms of infected sugar beet plants produced by SBYV owing to supposed lack of genotypic uniformity of the infected beet plants have been mentioned incidentally by Hull (1952) in reporting tolerance breeding experiments. Also Sedlag (1954) describes variations in the symptoms between different plants within a tolerant sugar beet variety. In most German investigations of SBYV, Schlösser's inbred sugar beet variety 9819 (pedigree: Schlösser 1952) has been used, which reacts with relatively uniform symptoms when infected with different isolates (e.g. Beiss 1956, Burghardt & Bercks 1959). However, also within this variety a certain variation of the symptoms can occur even if stable isolates of SBYV are used, for instance concerning the appearance of brown necrotic spots (dunkle Punkte) on the leaves of different plants (Bercks & Zimmer 1956).

II. PRESENT MATERIAL

Each year during 1946—1960 a number of field isolates of sugar beet yellows virus varying from 100 to 300 samples were compared in the greenhouse by infections of *Beta vulgaris* L. with *Myzus persicæ* (Sulz.) as vector. During 1946—57 mainly the Hilleshög commercial sugar beet variety was used; during 1958—60 two inbred sugar beet lines (Hilleshög 5788 and 5807) were added. Besides several of the virus isolates were tested on *Chenopodium foliosum* Aschers. in order to get a complementary check on their degree of severity. Serological tests and cross-protection experiments were made with some of the material (Björling 1958). Several isolates have been kept in culture through serial transfers for a long time; thus two from 1948, two from 1950 and many from the middle fifties still remain. These isolates have shown a high degree of stability in respect to the symptoms produced and they may be designated as strains according to the terminology of McKinney & Greeley (1960): "The term "strain" signifies an isolate, the characteristics of which remains essentially constant in serial transfers".

The different isolates were classified with the guidance of the symptoms of the test plants (commercial sugar beets, infected at the cotyledon stage) according to most or all of the following data:

1) incubation time and degree of vein-clearing of the first pair of leaves;
2) degree of vein-clearing and etch of the second and the following pair of leaves;
3) time of incipient formation of necroses on the first pair of leaves;
4) time of death of the first pair of leaves;
5) extent, distribution and tinge of the yellowing;
6) degree of stunting of the plants;
7) intensity of pin-points of the leaves;
8) occurrence of round brown spots on older leaves;
9) yield depression in roots and sugar content;
10) increase of noxious nitrogen in the roots.

With regard to the appearance of the symptoms according to 1)—6) above, the isolates could be roughly and fairly arbitrarily divided into the following four groups. However, the limits between the groups were by no means distinct; on the contrary the whole material seemed with respect to

the visual symptoms to represent a continuous series from very severe to very mild isolates.

Group a. Severe isolates. Incubation time for 1) was 7—12 days depending on light- and other growth conditions. Pronounced clearing of the veins, in some sugar beet varieties combined with a downwards directed rolling of the leaves. The second pair of leaves and some of the following leaves showed typical clearing of the veins and severe etch in varying degrees. These symptoms also appeared on test plants infected at later stages of development. Necroses occurred on the first pair of leaves 15—20 days after the infection and these leaves died some days later. Yellowing moderate, of less extent than in groups b, c and d. The diseased plants became distinctly stunted and yielded much less than plants infected by isolates from groups b, c and d. Visible differences occurred between different severe isolates. Thus three isolates of different origin belonging to this group have been compared in several tests during the last six years and they have throughout shown constant, minor differences.

Group b. Moderately severe or intermediate isolates. The incubation time for the symptoms 1)—5) was about one to two days longer than in group a. Distinct clearing of the veins on most test plants but not so pronounced as in group a. This symptom as a rule occurred clearly only on the first pair of leaves but now and then also on the second leaf-pair. No typical clearing of the veins on the following leaves but rather often moderate etch, particularly on the tips of the leaves. More pronounced yellowing, but less stunting than in group a. Conspicuous differences in the degree of severity between different isolates.

Group c. Mild isolates. The incubation time for the symptoms 1)—5) was some days longer than that of the preceding group. Faint clearing of the veins on the first pair of leaves, but often one or more of the infected test plants did not show this symptom. Sometimes indistinct etch on the tips of older leaves. Marked and extensive yellowing. Three different isolates belonging to this group have shown constant, minor differences in serial transfers during several years.

Group d. Very mild isolates. Characteristic of this group is the non-occurrence of vein-clearing and etch; the first visible symptom being yellowing of the first or the second pair of leaves three to six weeks after the infection. The isolates of this group are probably identical with sugar beet mild yellowing virus (SBYMV, Russel 1958, 1960), but this question is not yet settled. — The experiments described later in this paper were all made by isolates of the groups a, b and c.

Concerning the frequencies of the different groups of sugar beet yellows virus isolated during different years in Sweden it may be mentioned that during the end of the forties and the beginning of the fifties group b was predominant with single cases of group a and somewhat more cases of group c. It is possible that representatives of group d were also present during this time but were overlooked. However, if that is the case, group d would probably have been uncommon, as isolates belonging to groups a, b and c were at that time obtained from about 95 % of all leaf samples with yellows. From the middle fifties group d appeared in increasing frequencies in conjunction with a continuous general decrease of the incidence of the disease and in 1958 this group was predominant among the collected isolates. The following year (1959) was remarkable as isolates from group a and group b in about equal proportions made up the majority of the samples collected. Besides this fact could in respect to the occurrence of group a be established already by the symptoms in the fields, where the disease started late but swiftly reached an unusually high incidence. Next year (1960) the incidence of the disease was very low, possibly to some extent owing to extensive control measures against different infection sources (intensified clearing of clamped mangolds and increased sprayings of beet steckling- and beet seed crops), and the majority of the isolates collected belonged to group d.

III. PRODUCTION OF VEIN-CLEARING AND ETCH OF DIFFERENT VIRUS ISOLATES

While isolates belonging to group a (severe) regularly produced distinct vein-clearing and later etch on the infected test plants of the commercial sugar beet variety and isolates from group d (very mild) never produced these symptoms, it quite often happened that some of the positively reacting test plants infected by isolates from group b (intermediate) or c (mild) did not show typi-

Table I. COMPILED DATA FROM FIVE TRANSMISSION TESTS WITH INTERMEDIATE ISOLATES OF SBYV AND VARYING NUMBER OF APHIDS

Test plant: sugar beet, commercial variety

Number of Aphids per Test Plant	Result	Inf. %	Infected Plants with Vein-clearing	
			Number	%
1	37/140 a)	26	24	65
3	82/140	59	61	74
9	114/140	81	99	87

a) Numerator: number of plants infected; denominator: number colonized

cal vein-clearing or etch before yellowing. As different possibilities of explaining this irregular behaviour exist, e.g. heterogeneity of the virus isolate, inoculation of the virus at different sites of the plants or in varying amounts or else different genotypic response to the infection of the test plants as belonging to a cross-fertilizing species, some further experiments were carried out to study this question.

Three intermediate SBYV isolates were compared during 1953 and 1954 in five transmission experiments in which one, three and nine aphids (M.p.) were used on each test plant. Only slight and not significant differences in the percentage of infected plants with and without vein-clearing were in this case noted between the different isolates tested. The figures from the five experiments are compiled in Table I. Besides the higher number of infections obtained by higher number of aphids per test plant, a fact already known from earlier investigations (Watson 1946, Björling 1952), also the percentage of plants with vein-clearing was somewhat increased by higher number of aphids but not significantly so. — However, even with 9 aphids on each test plant 13 % of the infected plants did not show this symptom but developed yellowing directly some weeks after the infection. The average intensity of the vein-clearing was also slightly more pronounced on the test plants infected by nine aphids, but several of the plants infected by single aphids showed as strong symptoms as those infected by nine. In similar experiments Watson and Russel (1956) recorded much more marked differences between the intensities of the symptoms produced by many and by few aphids.

From test plants with and without vein-clearing obtained from one of the virus isolates in the above-mentioned experiment (Table I) two serial selective transfers with respect to the occurrence and non-occurrence of vein-clearing were made using one M.p. per test plant and varying test feeding times. Both types of virus sources produced vein-clearing and no vein-clearing on the new test plants and no tendency towards a selection of either symptom type was discernible (Table II). The low infection frequency in transfer 2 is probably due to the short test feeding time.

In order to study the occurrence of vein-clearing symptoms on genotypically more homogeneous beet test plants, 44 isolates of SBYV belonging to group b (moderately severe or intermediate) collected in different parts of North-western Europe were tested on an inbred sugar beet variety (9819), the seed of which was kindly delivered by Dr. Schlösser, Einbeck. The test was carried out during optimal light conditions (May-June 1953) with 3 M.p. on each test plant. The result was 277 infected from 320 inoculated plants. Of the infected plants 222 (80 %) showed typical vein-clearing. In 16 of the virus isolates all test plants (5 or 10) had this

Table II. SELECTIVE TRANSFERS FROM PLANTS WITH AND WITHOUT VEIN-CLEARING SYMPTOMS. ONE APHID ON EACH TEST PLANT

Test plant: sugar beet, commercial variety

Transfer No.	Symptoms of Source Plant	No. of Plants Analyzed	Test Feeding Time, Hours	Result	Proportion of Plants with Vein-clearing
1	No vein-clearing	7	24	30/70	19/30
	Vein-clearing	7	24	27/70	11/27
2	No vein-clearing	10	1/2	16/100	13/16
	Vein-clearing	7	1/2	14/70	9/14

Table III. COMPILED DATA FROM 35 COMPARATIVE EXPERIMENTS WITH TWO INTERMEDIATE ISOLATES OF SBYV. VARYING NUMBER OF APHIDS PER TEST PLANT

Test plant: sugar beet, commercial variety

Time	No. of Experiments	Virus Isolate	Result	Inf. %	Infected Plants with Vein-clearing		Average Inc. time Days
					Number	%	
March 1954 to Sept. 1954	23	48—511	151/215	70	139	92	12
		50—25	156/226	70	92	59	15
Sept. 1954 to March 1955	12	48—511	77/111	69	66	86	14
		50—25	74/128	58	34	46	16

symptom but in 28 isolates a varying number of the test plants developed yellowing directly without previous vein-clearing. In continued tests with the first-mentioned 16 isolates one or more of the infected test plants appeared without typical vein-clearing in all isolates on as well 9819 as the Hilleshög commercial variety but with a somewhat higher frequency of non-vein-clearing plants in the last-mentioned variety.

The ability to produce vein-clearing on commercial beet plants seems to be fairly different in different intermediate and mild isolates of SBYV and the occurrence of this symptom also varies with the light conditions. Thus two intermediate isolates kept in culture since 1948 and 1950 respectively were compared for different reasons on commercial sugar beets during 1954—55 in 35 green house experiments. Of these 23 were carried out during the lighter part of the year (21/3—20/9) and 12 during the darker part (21/9—20/3). In the last-mentioned ones additional light (mercury- and day light bulbs) was used (Table III).

During both parts of the year the differences in the frequency of vein-clearing were statistically significant between the two isolates. The average incubation times were also different but not significantly so between the two isolates and these times were somewhat prolonged during the winter. — In other tests without satisfactory additional light during the darker part of the year both the number of infected plants and the frequency of vein-clearing fell considerably below the values in Table III.

IV. ATTEMPTS TO SELECT MILD AND SEVERE TYPES FROM INTERMEDIATE ISOLATES

The fact pointed out in Section III that intermediate isolates of SBYV produce a varying degree of vein-clearing on plants of a commercial beet variety also affects the development and the intensity of the subsequent symptoms (2—6, p. 00). Thus if a sufficient number of plants is inoculated with such an isolate, a range of variation from relatively severe to fairly mild symptoms may be obtained, which gives an impression of heterogeneity of the virus isolate in question. Different intermediate isolates were, however, clearly separated from each other with reference to their average severity; while some produced moderately severe symptoms on most test plants and only few severe or mild symptoms, other isolates caused more intermediate and mild symptoms. Frequent attempts to select stable severe and mild types from such isolates of SBYV originally obtained from infected beet plants in the fields have failed.

An illustration of some of the unsuccessful attempts is shown in Fig. 1. Within two groups of commercial beet plants, each infected with one of the two intermediate virus isolates used in the experiments reported in Table III, six serial selective transfers were made during 1958 to new beet plants of the same variety from the plant with the most severe and from the plant with the most mild symptoms within each of the successive batches of test plants. One of the isolates (48—511) caused on the average more severe symptoms than the other one (50—25), but in both series plants with severe (s), intermediate (i) and mild (m) symptoms occurred on some occasions. The transfers from plants with the most severe and the most mild symptoms within each isolate showed the same range of symptom variation on the infected test plants and as in earlier attempts it was not possible to select stable types with more severe or more mild symptoms. In isolate 50—25 parts of the infections in transfers 3 and 4 failed and

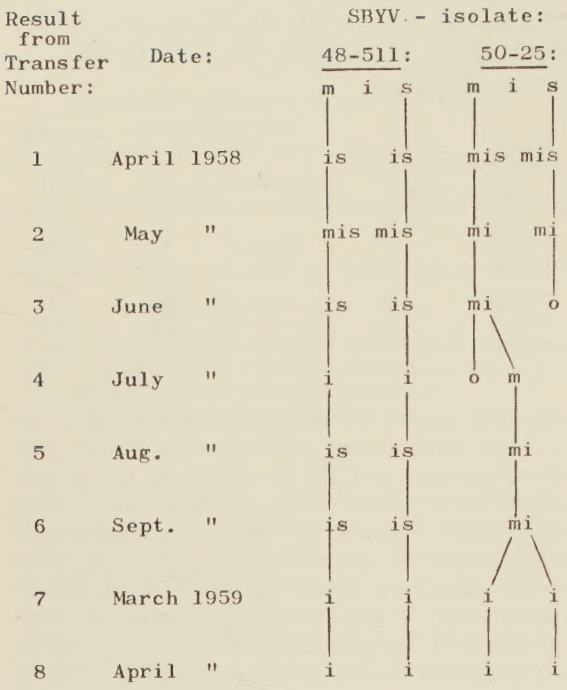
Result from Transfer Number:
Date:
SBYV. - isolate:
48-511:
50-25:
m i s
m i s
1 April 1958 is is mis mis
2 May " mis mis mi mi
3 June " is is mi o
4 July " i i o m
5 Aug. " is is mi
6 Sept. " is is mi
7 March 1959 i i i i
8 April " i i i i

Fig. 1. Attempts with negative results to secure divergent virus types by selective transfers from plants with the most severe and the most mild symptoms within groups of commercial sugar beets infected by two intermediate isolates of SBYV. The six first transfers were made on commercial beets; the two last ones on a highly inbred beet line. Symbols: m = plants with mild, i = with moderately severe or intermediate and s = with severe symptoms

therefore the series became incomplete for this isolate. Next year (1959) two more transfers were made, but to highly inbred beet plants (Hilleshög 5788), at which almost identical, intermediate symptoms were obtained on all infected test plants within each isolate independent of the symptoms of the virus source plants. The difference between the two isolates was visible; 48—511 was classified as moderately severe and 50—25 as intermediate-mild. The symptoms of the former were milder than those produced by a typically severe reference isolate and the symptoms of the latter were clearly more pronounced than those produced by a typically mild isolate used as another reference.

Fairly large variations in the average level of the intensity of the symptoms occurred between the different transfers. This was also observed in similar tests during other years. As a rule the most pronounced symptoms were obtained during March-May under the light conditions at Uppsala (day light and additional light). In experiments in the middle of the summer the symptoms were sometimes clearly inferior and in the autumn again better, but not so distinct as in the spring. Even experiments receiving the same total amount of light carried out in the spring and in the autumn with the same time relations to the vernal and the autumn equinox differed in this respect. This may be due to a favourable effect on the development of the symptoms of the increasing length of the day concurrent with the growth of the plants in the former case or to a reverse effect of the decreasing length of the day in the latter case or to both factors.

V. SYMPTOMS OF SBYV ON HIGHLY INBRED SUGAR BEET LINES

During 1958 a series of comparisons of the variation of the symptoms produced by a number of mostly intermediate isolates of SBYV was started on one hand on highly inbred sugar beets and on the other on the commercial sugar beet variety. At disposal were seven diploid sugar beet lines, inbred since about fifty years and four tetraploid, moderately inbred lines, the seeds of which were kindly delivered by Professor J. Rasmusson, Hilleshög. The pedigree of the diploid lines was as follows. The inbreeding began some time during the first decade of this century by Professor Hj. Nilsson at the Swedish Seed Association, Svalöv and the lines were multiplied by self-fertilization until the beginning of the thirties, when they were allowed to open pollination in separations during a succession of years. During this period the scarce, as a rule conspicuous outbreedings from normal beets were removed. From the middle forties onwards the multiplication was continued as strict self-fertilization. — The inbreeding of the tetraploid lines was moderate (three or four generations).

1. *Variations of the symptoms within different SBYV isolates*

In each comparative experiment groups of test plants (10—20 per group) partly of one or some diploid and tetraploid inbred lines, partly of commercial beets were infected by different isolates of SBYV. In five out of eleven experiments carried out during April-Sept. 1958 with fifteen different virus isolates, two groups of test plants of the same kind were infected from two different virus

sources of certain intermediate isolates. Two infected plants of the commercial variety served as virus sources in these cases, one with the most severe and one with the most mild symptoms produced within the isolates in question. In all these cases the same symptoms were obtained on both groups of test plants of the same kind infected with the same isolate independently of the symptoms of the two virus source plants. These observations confirm the results obtained in the serial transfers reproduced in Fig. 1. — In order to get as similar infection conditions as possible beet clone plants infected with the different isolates (Section VI) were used as virus sources in six of the eleven experiments in 1958.

The variation of the symptoms within the groups of test plants of the commercial variety infected by intermediate isolates was considerable and it ranged as was earlier observed within some isolates from relatively severe to more or less mild symptoms. On the infected test plants of the diploid inbred lines very uniform, often almost identical symptoms were obtained by all isolates tested. This was especially the case when each plant was colonized by three or more infective aphids (M.p.). However, some minor variations of the length of the incubation time were noted also on the inbred plants and this variation was somewhat greater when only one aphid was used on each test plant. Such a prolongation of the incubation time may involve that on plants infected at the cotyledon stage, vein-clearing and etch will appear only on the second pair of true leaves and yellowing and necroses will be retarded some days. As a rule such plants will be less stunted than those with a normal incubation time, but in spite of this after five-six weeks the general symptoms of all diploid inbred plants infected by the same virus isolate were very uniform. A similar prolongation of the incubation time combined with less stunting in individual plants especially when only one aphid infects, also occurs in commercial beets and other test plants (e.g. *Tetragonia expansa* Murr., *Chenopodium foliosum* Aschers. and *Claytonia perfoliata* Donn.). The possible causes of this type of symptom variation are being studied in special experiments, which will be discussed in another paper.

The symptoms produced on the inbred tetraploid sugar beet lines were within each virus isolate much more uniform than those on the commercial variety, but markedly less uniform than those on the diploid inbred lines. This might be interpreted as a manifestation of a lack of genotypic homogeneity within the tetraploid lines in consequence of a considerably less degree of inbreeding.

2. *Variations of the symptoms between different SBYV isolates*

The differences in the degree of severity of the symptoms produced by different virus isolates were large on all tested inbred sugar beet lines and especially prominent between the groups severe, intermediate and mild. Within each of these three groups of isolates the differences between the symptoms produced by different isolates were less, but often distinct, which to a certain extent appears from Fig. 2, where leaves from an inbred diploid line infected by a severe, three intermediate and a mild SBYV isolate are reproduced. However, the possibilities of differentiating very similar, but still different isolates of SBYV are limited even by as highly inbred sugar beet lines as the present diploid ones. This is evident for instance from the observation made more than once that moderately severe isolates, which were clinically almost identical on one or several inbred sugar beet lines, in tests on *Chenopodium foliosum* produced slightly different symptoms and by weighing the plants in these tests small but significative differences between the yield depressions were recorded.

3. *Variations of the symptoms between different inbred sugar beet lines*

Evident differences of the symptoms were observed between some but not between all of the inbred diploid sugar beet lines infected by one and the same virus isolate. Among other things these differences referred to the tinge and the distribution of the yellowing; some lines became more orange-coloured by intermediate and mild isolates; in some lines infected by some intermediate isolates the yellowing was more flamed than in other lines. Furthermore the tendency to produce so-called pin-points was clearly different in different inbred sugar beet lines. This symptom, which seems to appear most distinctly in the autumn on middle-aged leaves four to six weeks after the infection (Fig. 3), has been reported previously by different authors (Schlösser 1952, Bercks & Zimmer 1956, Bennet 1960, Esau

Fig. 2. Symptoms of different isolates of SBYV on leaves from the second leaf-pair of a highly inbred sugar beet line. A: severe isolate; pronounced etch. B, C, D: three different intermediate isolates; varying degrees of etch, mostly in the tips of the leaves, in D more intensive yellows. E: mild isolate; only yellows. F: leaves from healthy plants

1960). It is true that only a small part of all virus isolates collected during the present study were tested under growth conditions especially favourable for the appearance of this symptom, but the impression accumulated from several observations is that probably all isolates of true SBYV are able to produce pin-points in a varying degree and that the extensiveness of this symptom is determined both by the inherent disposition of the host genotype and the virus isolate present. This interaction is indicated by the heterogeneous reaction of commercial sugar beets infected by a separate virus isolate: some plants may show clear pin-points, some indistinct ones and some none, but especially by the fact that obvious differences in this respect exist between different inbred sugar beet lines and also between different beet clones (Table IV). On the other hand some SBYV isolates regularly produce more distinct and more numerous pin-points than other isolates and broadly speaking a positive correlation seems to exist between general severity and ability to develop pin-points. However, exceptions occur; two intermediate isolates that were compared for some years with a number of severe, intermediate and mild ones, produced throughout more numerous and more marked pin-points than the rest of the material.

The differences of the symptoms between

Fig. 3. Pronounced pin-point reaction produced by an intermediate isolate of SBYV

the four inbred tetraploid sugar beet lines infected by the same virus isolate were clearly greater than between the diploid ones and referred to the tinge and extent of the yellowing, to the degree of stunting and to the occurrence of dark brown round necrotic spots with one to two mm. diameter on older leaves. The last-mentioned symptom that seems to be similar to but not identical with the necrotic spots on diploid sugar beet leaves described by Bercks & Zimmer (1956), was produced in a somewhat varying degree by the virus isolates tested in this series, but to a very different extent on the different tetraploid beet lines.

VI. SYMPTOMS OF SBYV ON BEET CLONES

In order to compare the effects of infections by different SBYV isolates on a genetically wholly homogeneous beet material a number of beet clones were produced. Large roots of field plants partly from commercial varieties, partly from breeding material were selected in the autumn; after defoliation and storage the roots were planted in a greenhouse during Jan. or Febr. and three to four weeks later the growing shoots were excised with appendant, about one cm^3 large, cube-shaped pieces of the tissue of the mother-root. These stecklings were treated with napthyl-acetic acid for 24 hours and planted in sand that was watered with nutrient solution. The formation of new roots varied much between the groups of stecklings from different mother-roots. Maximally about 35 plants fit for use were obtained directly in this manner from each of some sugar beet roots; the average number of plants per clone came up to 25, but some mother-roots delivered only about 10 plants. Some weeks before the infection, which as a rule could be made in April-May, the clone plants were trimmed to the same size. Within each clone groups of two to four plants were infected by different virus isolates as well in the greenhouse as in the field. During 1954—1960 inclusive, 38 different sugar beet clones and 10 different mangold clones were exploited in infection experiments; some clones were used during some years in succession by renewed production of stecklings each year.

Essentially the same result concerning the symptoms produced by different virus isolates was obtained as in the experiments with highly inbred diploid sugar beet lines. Thus different plants within the same clone infected by a separate SBYV isolate reacted with very similar, almost identical symptoms. The differences of the symptoms produced by different virus isolates were pronounced within the same clone concerning as well vein-etch and yellowing as the degree of stunting (Fig. 4). Between some of the morphologically often very dissimilar clones (Fig. 5), obvious differences of the symptoms produced by the same SBYV isolate occurred,

Fig. 4. Different degrees of yellows, etch and stunting produced by different isolates of SBYV on plants of beet clone 35. The plants were infected in pairs; the two plants to the right in the uppermost row not infected. Sept. 1959

especially concerning the extent and the tinge of the yellowing, but also in the degree of stunting. The relative yield depressions caused by separate virus isolates were in some cases clearly different in different beet clones, but statistically significant values of these differences were not obtained owing to the small number of infected plants and to the considerable variation in root size also in uninfected clone plants.

The tendency to form pin-points was differently pronounced in different beet clones. Within some clones infected by different virus isolates this symptom was not observed. On other clones visible pin-points appeared after infection by some, preferably intermediate and severe isolates but not after infection by other intermediate and mild isolates. On some clones pin-points were produced by all isolates tested, but in a varying degree. Some comparisons in the greenhouse partly between plants belonging to different clones eight months after infections by a number of SBYV isolates partly with commercial beet plants of equivalent age and infection time are shown in Table IV. The intensity of the pin-point reaction was graded in 0, trace (tr.), 1, 2 or 3. The last-mentioned intensity corresponds to Fig. 3. In all cases the symptoms were very uniform on all plants within the same clone infected by a separate virus isolate, while variations from 0 to 3 occurred within the corresponding groups of plants of the commercial variety. The same tendency to a positive correlation between the general severity of the isolates and their ability to produce pin-points as was observed in the infection experiments with inbred diploid lines, occurred at infections of clones that reacted in any appreciable degree. However, some intermediate isolates e.g. i 2 in Table IV, that in other respects (vein-etch, necroses, stunting etc.) were clearly milder than the severe isolates, produced quite as many pin-points as the last-mentioned ones.

Although infection experiments with beet clone plants produced according to the method applied are impaired by the disadvantage of large variations in root weight

Fig. 5. Rows of different beet clones; three plants in succession in each row infected by a separate isolate of SBYV. August 1957

also in uninfected plants, virus-infected beet clones yet afford important advantages in certain respects owing to the possibility of introducing and keeping different virus isolates for a long time in the same host plant genotype. Such infected clone plants are suitable for instance as virus sources at repeated comparisons between different isolates or between different vectors (Björling & Ossiannilsson 1958 b), and they have also been used in paper-electrophoretic and electron-microscopical studies and as sources of antigens in antiserum production.

VII. DISCUSSION

With the transmission technique applied, the stability of the described isolates of SBYV was very high. Some isolates have been kept in culture through many transfers for more than ten years and several for six to eight years without their characteristics being changed. They may consequently be regarded as strains according to the definition proposed by McKinney & Greeley (1960). All attempts to select and breed types with deviating symptoms from groups of plants of a commercial sugar beet variety showing varying symptoms after infection by one of these virus strains have failed. Attempts to secure divergent virus types by transferring some of the strains through other susceptible plants have also been negative; the characteristics of the original strains were not changed by this treatment (Björling 1958). As the last-mentioned experiments contained only a single passage of the virus strains through other host plant species, extended tests were carried out during Nov. 1959 to May 1960 comprising five serial transfers of three different, well-defined SBYV strains through *Beta vulgaris* L., *Tetragonia expansa* Murr., *Chenopodium foliosum* Aschers., *Claytonia perfoliata* Donn. and *Comphrena globosa* L. In some of the fifteen strain-host combinations the virus was lost, but isolates from the three strains passed in all transfers besides through the beets, through at least two of the other host plant species. Careful comparisons of the virus material left at the termination of the series by further infection experiments on beets, *T. expansa* and *Ch.*

Table IV. INTENSITY OF PIN-POINTS PRODUCED BY DIFFERENT ISOLATES OF SBYV ON COMMERCIAL SUGAR BEETS AND DIFFERENT BEET CLONES. THE REACTION GRADED IN 0, TRACE (tr.), 1, 2 OR 3

Date of recording Symptoms	No. of Plants inf. with each Isolate	Type of Plants	Type of Virus Isolate: Mild:	Intermediate:				Severe:		
			m 1	m 2	i 1	i 2	i 3	i 4	s 1	s 2	s 3
Jan. 1957	5	Comm. beets	0, tr., 1	0, tr.	0, tr., 1				0, 1, 3	0, 1, 2	
„ „	4	Clone 14	tr.	0	2				2	3	
Jan. 1960	3	Clone 38	1			3	2	1		3	
„ „	3	Clone 40	0			2	1	tr.		2	
„ „	2	Clone 43	0	1	2					3	2

foliosum showed that no visible changes of the characteristics of the three SBYV strains had been brought about in consequence of the passages through other hosts than sugar beets.

Some of the virus strains especially mild and very mild ones were lost in the course of the years owing to abortive transfers. However, this need not indicate a genetic instability of the strains in question, but is rather due to defects of the general transmission technique. According to serological tests too long intervals between the transfers during the winter often result in a very low concentration of active virus especially in older plants, probably so low that the vectors do not become infective. The only distinct cases of instability within the SBYV material studied were found among the progeny from simultaneous mixed infections with two very different strains, where besides the two original strains also intermediate types appeared in the subsequent transfers. The latter types are being studied since some years in serial transfers in order to determine whether they are more or less firm mixtures of the original strains or new stable strains produced by recombination.

The observed stability through many serial transfers of the SBYV strains isolated from infected field plants does not exclude the possibility of the formation of mutants within the strains in question. If that is the case, such mutants probably are quickly eliminated by being overgrown by the mother strain. Moreover a successful separation of any slower-growing variants that may occur, does not seem achievable by the aid of vector-transmissions as it is probable that a large number of virus particles are transferred at each transmission even if only a single vector specimen is used. However, in view of the natural occurrence of a large number of different SBYV strains, some sort of mechanism responsible for the genetic variation within this virus must exist. — In all plant viruses that have been studied more closely, different strains have been found with very few exceptions, for instance tomato bushy stunt virus (Bawden & Roberts 1947). In some cases as in TMV and potato virus X variants have been secured by different methods from strains under experimental conditions that indicate that the new variants have arisen as mutants (Knight 1959). However, within several other plant viruses experimental evidence for the formation of certain mutants has not yet been established in spite of the occurrence of many strains. High stability of different strains of sugar beet curly top virus was recently reported by Giddings (1959).

The variations of the symptoms of SBYV that occur on sugar beets in infection experiments especially within commercial varieties also when a stable virus strain is used may have different causes. It is well known that partly various environmental factors, especially the light conditions, partly the developmental stage of the plants at the time of the infection in a high degree affect the intensity of the symptoms. The variations caused by the environmental factors are valid primarily between different experiments carried out at different times and with reference to the developmental stage this is easily controlled within and between different experiments. As to the variation of the symptoms within one and the same experiment and between plants infected by a stable virus strain, this variation seems to be partly genetically conditioned and caused by the genotypic variation of the host plants when commercial varieties are infected and

partly phenotypic, probably caused by the mode of infection and possibly also to some extent by different growth rates of the infected plants. That fairly different symptoms can be produced by a stable SBYV strain on different plants of a commercial sugar beet variety is in itself not surprising considering the considerable genetical variation within such a variety. Similar symptom variations in virus infections of another naturally cross-pollinating species viz. Ladino clover *(Trifolium repens* L.) infected by alfalfa mosaic and bean yellow mosaic viruses have recently been described, in which case the variations of the symptoms came into view most clearly at infections of clonal lines (Kreitlow et al. 1957, Kreitlow & Hunt 1958). Furthermore it may be pointed out that a still larger host-conditioned symptom variation occurs between different varieties (= clones) of *Solanum tuberosum* L. infected by potato virus Y, in which case the infections as is well known may result in clinically very different diseases.

Also in a genetically homogeneous host-virus combination, for instance in the experiments with highly inbred sugar beet lines infected by stable SBYV strains (section V), some apparently phenotypic variation of the symptoms occurs, especially if only a single vector specimen is used on each test plant. This variation principally manifests itself as a prolongation of the incubation time in some plants, which involves somewhat milder symptoms, particularly less stunting. Cases have also been reported when many viruliferous aphids per test plant produce more severe symptoms of sugar beet yellows than few aphids do (Watson & Russel 1954, 1956, Bercks & Burghardt 1958, Steudel 1958). Whether this variation reflects an effect of the amount of virus initially inoculated in the plants as surmised by Watson & Russel (1954) and Steudel (1958), or if the site or the number of sites of the infection on the plant referring to different leaves or different tissues is of crucial importance, is still an open question.

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